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Glenn P. Ladwig, Patent Attorney, Reg. No. 46,853

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR 1.322
Docket No. USF.183XC1
Patent No. 7,655,772

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Shyam S. Mohapatra
Issued	:	February 2, 2010
Patent No.	:	7,655,772
Conf. No.	:	4246
For	:	Materials and Methods for Treatment of Allergic Diseases

Mail Stop Certificate of Corrections Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 37 CFR 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

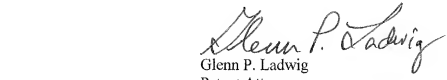
Patent Reads:	**Application Reads:**
Column 1, line 50:	Page 2, line 2:
"tissue Needleham, P."	--tissue (Needleham, P.--
Column 3, line 60:	Page 5, line 6:
"itranasally."	--intranasally.--
Column 4, lines 12-13:	Page 5, line 19:
"methachohne."	--methacholine.--
Column 4, line 45:	Page 6, line 8:
"means±SEM"	--means ±SEM--
Column 9, line 29:	Page 10, line 13:
"vsntdhndfk"	--vsntdlmdfk--
Column 10, line 29:	Amendment Under 37 CFR § 1.111 dated May 11, 2009, page 2:
"ttg aaa ctg"	--ttg aaa agc aaa ctg--
Column 13, line 50, Table 1, row "Uncharged Polar":	Page 15, line 2, Table 1, row "Uncharged Polar:
"Gly, Ser, Thr, Cys, Tyr, Asn, Gin"	--Gly, Ser, Thr, Cys, Tyr, Asn, Gln--
Column 14, lines 13-14:	Page 15, line 20:
"SLAM J. Applied Math.,"	--SIAM J. Applied Math.,--
Column 15, line 39:	Page 17, line 26:
"DLMDFKMLLDHL"	--DLMDFKNLLDHL--
Column 15, line 40:	Page 17, line 27:
"DLMDFKMLLDHL"	--DLMDFKNLLDHL--

Column 16, line 5:	Page 18, line 15:
"fimgi cells"	--fungi cells--
Column 17, line 31:	Page 20, line 1:
"co-admistered"	--co-administered--
Column 18, line 1:	Page 20, line 27:
"Small unilarnellar vesicle"	--Small unilamellar vesicle--
Column 18, line 11:	Page 21, line 2:
"nanometers (mn),"	--nanometers (nm),--
Column 21, line 8:	Page 25, lines 7-8:
"(e.g. a protein,"	--(*e.g.*, a protein,--
Column 26, line 6:	Page 32, line 8:
"descnbedpreviously"	--described previously--
Column 26, line 30:	Page 32, line 24:
"365 mn"	--365 nm--
Column 26, line 48:	Page 33, line 5:
"N_{ω}-nitro-L-arginie"	--N_{ω}-nitro-L-arginine--
Column 26, line 53:	Page 33, line 9:
"inportant"	--important--

A true and correct copy of pages 2, 5, 6, 10, 15, 17, 18, 20, 21, 25, 32, and 33 of the specification as filed and the Amendment Under 37 CFR § 1.111 dated May 11, 2009 which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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GPL/jnw

Attachments: Copy of pages 2, 5, 6, 10, 15, 17, 18, 20, 21, 25, 32 and 33 of the specification
Copy of Amendment Under 37 CFR § 1.111 dated May 11, 2009

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,655,772

APPLICATION NO.: 10/526,584

DATED : February 2, 2010

INVENTOR : Shyam S. Mohapatra

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Line 50, "tissue Needleham, P." should read --tissue (Needleham, P.--.

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Column 14,
Lines 13-14, "SLAM J. Applied Math.," should read --SIAM J. Applied Math.,--.

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

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Line 53, "inportant" should read --important--.

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The C-terminal peptide of proANF (also known by the synonym proANP), ANP is a 28-amino acid hormone secreted by the cardiac atria and lung tissue (Needleham, P. *et al.*, *N Engl J Med*, 1986, 314:828-834). ANP has vasodilator, natriuretic and diuretic properties (Needleham, P. *et al.*, *N Engl J Med*, 1986, 314:828-834). ANP infused at high concentrations reduces airway resistance in normal subjects (Hulks G. *et al.*, *Clin Sci* 1990;79:51-55) and produces a significant bronchodilator response in patients with asthma. Inhaled ANP attenuates histamine- and methacholine (MCh)-induced bronchoconstriction (Hulks, G. *et al.*, *Br. Med J*, 1992, 304:1156; Angus, R.M. *et al.*, *Clin Exp Allergy*, 1994, 24:784-788); however, the amount of ANP required for efficacy and their short half-life limits their use for long-term modulation of airway hyper-responsiveness (Harnet, P. *et al.*, *Nephrologie*, 1987, 8:7-12; Matsuse, H., *et al.*, *J Immunol*, 2000, 164:6583-6582).

The present inventor has demonstrated prolonged amelioration of symptoms associated with respiratory allergy and asthma by delivery of pDNA-encoding various natriuretic hormone peptides (NHPs), or by delivery of the peptides themselves, which exhibit bronchodilatory and/or anti-inflammatory activity.

Brief Summary of the Invention

The present invention pertains to a method for treating respiratory allergies, such as allergic rhinitis and asthma, which may be caused by allergens and exacerbated by respiratory viral infections, pollutants, and smoke.

In one embodiment, the method of the present invention comprises administering a therapeutically effective amount of a natriuretic hormone peptide (referred to herein as NHP or NHP peptide) to a patient in need of such treatment. As used herein, NHP refers to atrial natriuretic factor (ANF) hormone, or a biologically active fragment or homolog thereof.

Specifically exemplified NHPs comprise an amino acid sequence selected from the group consisting of amino acids 1-30 of ANF (also known as "long acting natriuretic peptide" and referred to herein as NHP_{1-30} or SEQ ID NO:1), amino acids 31-67 of ANF (also known as "vessel dilator" and referred to herein as NHP_{31-67} or SEQ ID NO:2), amino acids 79-98 of ANF (also known as "kaliuretic peptide" and referred to herein as NHP_{79-98} or SEQ ID NO:3), and amino acids 99-126 of ANF (also known as "atrial natriuretic peptide" or "ANP", and referred to herein as NHP_{99-126} or SEQ ID NO:4), or

exhibited higher titers ($p<0.01$) of ova specific IgE than the PBS control. The experiments were repeated twice and data from a representative experiment are shown. Figure 3D-E show the measurement of AHR to increasing concentrations of methacholine following NHP gene transfer on day 26. BALB/c mice (n=4) were sensitized with ovalbumin by i.p. immunization (10 μg/mouse) and 14 days later were treated with 10μg/mouse of SEQ ID NO:13 or $pNHP_{73-102}$ intranasally. The control group received the empty vector alone. Each mouse was intranasaly administered three times on two days interval with 10μg of plasmid DNA complexed with 50 μg of transfection reagent Lipofectamine (Life Technologies, Rockville, MD). Animals were challenged with the same allergen (50μg in PBS) three days after the last intransal DNA delivery and 24 hours later their AHR was measured using the whole body plethysmograph (Buxco, Troy, NY). A dose-dependent decrease of methacholine response is shown in Figure 3D. Figure 3E shows the effect of treatment with SEQ ID NO:13 and $pNHP_{73-102}$ on allergen-induced airway hyper-responsiveness (AHR). The effect of treatment at the highest concentration (50mg/ml) of methacholine challenge is shown ($p<0.05$).

Figures 4A and 4B show the long-term effect on AHR following prophylaxis by NHP_{73-102} gene transfer. Figure 4A shows schematically the protocol of sensitization, treatment and antigen challenges and measurement of AHR. Figure 4B shows measurement of Penh (%) at 50mg/ml of methacholine. *$p<0.05$; compared to pVAX control. The experiment was repeated twice and data from a representative experiment are shown.

Figures 5A-5C show that administration of chitosan-pNHP nanoparticles exhibit a therapeutic effect for allergen-RSV induced asthma and reversal of asthma in mice. Figure 5A shows an experimental outline of immunization protocol with allergen and RSV, treatment schedules, challenges and AHR measurements. Figure 5B shows reversal of airway hyper-reactivity as evident from % Penh measurement following treatment with chitosan + $pNHP_{73-102}$. The other treatments include chitosan + pVAX (control), chitosan + NHP_{73-102}, fluticasone, and fluticasone and salmeterol alone. Figure 5C shows the reduction in inflammatory cells in the lung by treatment with $pNHP_{73-102}$. Mice treated as shown in Figure 5B were subjected to bronchioalveolar lavage (BAL) following AHR. A BAL cell differential was performed and cytospun BAL cells were stained and different

cell types were quantified by three blinded investigators. The percentage of cells of macrophages, eosinophils, neutrophils and lymphocytes were determined.

Figures 6A-6C show that overexpression of NHP_{73-102} leads to increased production of nitric oxide in human epithelial cells. A549 (Figure 6A) and NHBE (Figure 6B) cells were transfected with control vector or NHP_{73-102}. At the indicated times after transfection, aliquots of the culture medium were assayed for nitrite (the NO reaction product). Fluorescence was read at 409 nm with excitation at 365 nm using a JASCO spectrofluorometer. Data are means ±SEM (n = 3). Figure 6C shows that NO production is due to the constitutive NOS. One aliquot of cells was incubated during the expression phase with 1 mM N_{ω}-nitro-L-arginine methyl ester, an arginine analog that blocks cNOS production of NO (NHP+i). The enhanced NO generation was inhibited by pretreatment of the cells with N-nitro-L-arginine methyl ester, which blocks cNOS activity.

Figures 7A and 7B show that $pNHP_{73-102}$ exerts its anti-inflammatory activity in the lung by decreasing NFκB activation in epithelial cells. A549 (Figure 7A) or NHBE (Figure 7B) cells were co-transfected with $pNHP_{73-102}$ or vector pVAX (pV) alone, NFκB plasmid carrying the luciferase reporter gene (pNFκB-luc reporter plasmid) (MERCURY PROFILING SYSTEM, CLONTECH), and pLacZ normalization control. NFκB was activated 24 hr after transfection by incubating cells with 20 ng/ml phorbol myristoyl acetate (PMA) (for A549 cells) or 10ng/ml of TNF-α (for NHBE cells). Luciferase activity was detected using the DUAL LUCIFERASE REPORTER Assay kit (CLONTECH) and DYNEX MLX luminometer. Data (average of three readings± SEM) are expressed as fold change in luciferase activity in arbitrary units relative to vector control.

Brief Description of the Sequences

SEQ ID NO:1 is the amino acid sequence of human "long acting natriuretic peptide" or NHP_{1-30}: ^{1}NPMYN AVSNADLMDF KNLLDHLEEK MPLED^{30}.

SEQ ID NO:2 is the amino acid sequence of human "vessel dilator" or NHP_{31-67}: ^{31}EVVPP QVLSEPNEEA GAALSPLPEV PPWTGEVSPA QR^{67}.

2041 taagtgaaat ttactctgat gagtaacttg cttatcaatt catgaagctc agagggtcat
2101 caggctgggg tgggggccgg tgggaagcag gtggtcagta atcaagttca gaggatgggc
2161 acactcatac atgaagctga cttttccagg acagccaggt caccaagcca gatatgtctg
2221 tgttctcttt gcagtactga agataacagc cagggaggac aagcagggct gggcctaggg
2281 acagactgca agaggctcct gtcccctggg gtctctgctg catttgtgtc atcttgttgc
2341 catggagttg tgatcatccc atctaagctg cagcttcctg tcaacacttc tcacatctta
2401 tgctaactgt agataaagtg gtttgatggt gacttcctcg cctctcccac cccatgcatt
2461 aaattttaag gtagaacctc acctgttact gaaagtggtt tgaaagtgaa taaacttcag
2521 caccatggac agaagacaaa tgcctgcgtt ggtgtgcttt ctttcttctt gggaagagaa
2581 ttc

SEQ ID NO:16 is the amino acid sequence for the mouse preproANP peptide
1 mgsfsitlgf flvlafwlpg higanpvysa vsntdlmdfk nlldhleekm pvedevmppq
61 alseqtecag aalsslpevp pwtgevnppl rdgsalgrsp wdpsdrsall ksklrallag
121 prslrrsscf ggridrigaq sglgcnsfry rr

SEQ ID NO: 17 is the genetic sequence for the mouse preproANP peptide wherein the coding sequence starts at nucleic acid molecule position 81 and ends at nucleic acid molecule position 539.

1 caaaagctga gagagagaga gaaagaaacc agagtgggca gagacagcaa acatcagatc
61 gtgccccgac ccacgccagc atgggctcct tctccatcac cctgggcttc ttcctcgtct
121 tggcctttg gcttccaggc catattggag caaatcctgt gtacagtgcg gtgtccaaca
181 cagatctgat ggatttcaag aacctgctag accacctgga ggagaagatg ccggtagaag
241 atgaggtcat gccccgccag gccctgagtg agcagactga ggaagcaggg gccgcactta
301 gctccctccc cgaggtgcct ccctggactg gggaggtcaa cccacctctg agagacggca
361 gtgctctagg gcgcagcccc tgggacccct ccgatagatc tgccctcttg aaaagcaaac
421 tgagggctct gctcgctggc cctcggagcc tacgaagatc cagctgcttc gggggtagga
481 ttgacaggat tggagcccag agtggactag gctgcaacag cttccggtac cgaagataac
541 agccaaggag gaaaaggcag tcgattctgc ttgagcagat cgcaaaagat cctaagccct
601 tgtggtgtgt cacgcagctt ggtcacattg ccactgtggc gtggtgaaca ccctcctgga
661 gctgcggctt cctgccttca tctatcacga tcgatgttaa atgtagatga gtggtctagt
721 gggtcttgc ctctcccact ctgcatatta aggtagatcc tcacccttt cagaaagcag
781 ttggaaaaaa aaaaaaagaa taaacttcag caccaaggac agacgccgag gccctgatgt

Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

Both protein and nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA, and CLUSTALW (Pearson and Lipman *Proc. Natl. Acad. Sci. USA*, 1988, 85(8):2444-2448; Altschul *et al. J. Mol. Biol.*, 1990, 215(3):403-410; Thompson *et al. Nucleic Acids Res.*, 1994, 22(2):4673-4680; Higgins *et al. Methods Enzymol.*, 1996, 266:383-402; Altschul *et al. J. Mol. Biol.*, 1990, 215(3):403-410; Altschul *et al. Nature Genetics*, 1993, 3:266-272).

Identity and similarity of related nucleic acid molecules and polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988;York (1988); Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993;York (1993); Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994;Jersey (1994); Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991;York (1991); and Carillo *et al.*, SIAM J. Applied Math., 48:1073 (1988).

The methods, pharmaceutical compositions, and vectors of the present invention can utilize biologically active fragments of nucleic acid sequences encoding the 126-amino acid atrial natriuretic factor (ANF) prohormone, such as nucleic acid sequences encoding NHP_{1-30}, NHP_{31-67}, NHP_{79-98}, NHP_{99-126}, and NHP_{73-102}, (SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5, respectively), SEQ ID NO:6, and including biologically active fragments of the nucleic acid sequences encoding SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

acids, preferably at least 12 amino acids, and still more preferably at least 15 or at least 20 consecutive amino acids of the polypeptide sequence from which it is derived. The upper limit for such fragments is one amino acid less than the total number of amino acids found in the full-length sequence.

In other embodiments, fragments of the polypeptides can comprise consecutive amino acids of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, and up to one amino acid less than the full-length ANF prohormone. Fragments of polypeptides can be any portion of the full-length ANF prohormone amino acid sequence (including human or non-human mammalian homologs of the ANF prohormone) that exhibit biological activity, *e.g.*, a C-terminally or N-terminally truncated version of the ANF prohormone, or an intervening portion of the ANF prohormone. In some embodiments, fragments comprise biologically active fragments of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

The present invention can be practiced using other biologically equivalent forms of ANF fragments or homologs thereof as can be appreciated by the sequence comparison below. Sequence similarities between mouse and human forms of ANP are shown where areas of conservation are clearly seen.

NCBI BLAST Comparison of mouse (Query) to human (Sbjct) ANP a.a. sequences.

```
Query: 1   MGSFSIT-LGFFLVLAFWLPGHIGANPVYSAVSNTDLMDFKNLLDHLEEKMPVEDEVMPP
           M SFS T + F L+LAF L G   ANP+Y+AVSN DLMDFKNLLDHLEEKMP+EDEV+PP
Sbjct: 1   MSSFSTTTVSFLLLLAFQLLGQTRANPMYNAVSNADLMDFKNLLDHLEEKMPLEDEVVPP

Query: 60  QALSEQTEEAGAALSSLPEVPPWTGEVNPPLRDGSALGRSPWDPSDXXXXXXXXXXXXX
           Q LSE  ERAGAALS LPEVPPWTGEV+P  RDG ALGR PWD SD
Sbjct: 61  QVLSEPNEEAGAALSPLPEVPPWTGEVSPAQRDGGALGRGPWDSSDRSALLKSKLRALLT

Query: 120 GPRSLRRSSCFGGRIDRIGAQSGLGCNSFRY 150
           PRSLRRSSCFGGR+DRIGAQSGLGCNSFRY
Sbjct: 121 APRSLRRSSCFGGRMDRIGAQSGLGCNSFRY 151
```

The NHP of the invention can be prepared by well-known synthetic procedures. For example, the polypeptides can be prepared by the well-known Merrifield solid support method. See Merrifield (1963) *J. Amer. Chem. Soc.* 85:2149-2154 and Merrifield (1965) *Science* 150:178-185. This procedure, using many of the same chemical reactions and blocking groups of classical peptide synthesis, provides a growing peptide chain anchored by its carboxyl terminus to a solid support, usually cross-linked polystyrene or styrenedivinylbenzene copolymer. This method conveniently simplifies the number of procedural manipulations since removal of the excess reagents at each step is effected simply by washing of the polymer.

Alternatively, these peptides can be prepared by use of well-known molecular biology procedures. Polynucleotides, such as DNA sequences, encoding the NHP of the invention can be readily synthesized. Such polynucleotides are a further aspect of the present invention. These polynucleotides can be used to genetically engineer eukaryotic or prokaryotic cells, for example, bacteria cells, insect cells, algae cells, plant cells, mammalian cells, yeast cells or fungi cells for synthesis of the peptides of the invention.

For purposes of the present invention, the biological activity attributable to the homologs and fragments of NHP and NHP-encoding nucleic acid sequences means the capability to prevent or alleviate symptoms associated with allergic disease, such as bronchoconstriction and inflammation. This biological activity can be mediated by one or more of the following mechanisms: increased production of intracellular Ca^{++} concentration (*e.g.*, in epithelial cells), increased production of nitric oxide (NO), and decreased activation of NFkB.

The methods of the subject invention also contemplate the administration of cells that have been genetically modified to produce NHP, or biologically active fragments thereof. Such genetically modified cells can be administered alone or in combinations with different types of cells. Thus, genetically modified cells of the invention can be co-administered with other cells, which can include genetically modified cells or non-genetically modified cells. Genetically modified cells may serve to support the survival and function of the co-administered cells, for example.

The term "genetic modification" as used herein refers to the stable or transient alteration of the genotype of a cell of the subject invention by intentional introduction of exogenous nucleic acids by any means known in the art (including for example, direct transmission of a polynucleotide sequence from a cell or virus particle, transmission of

sequences and pharmaceutical compositions of the invention can be co-administered (concurrently or consecutively) to a patient with other therapeutic agents useful for treating airway reactivity, airway inflammation, and airway remodeling.

Expression vectors for NHP are any which are known in the art that will cause expression of NHP-encoding nucleic acid sequences in mammalian cells. Suitable promoters and other regulatory sequences can be selected as is desirable for a particular application. The promoters can be inducible or tissue specific as necessary. For example the cytomegalovirus (CMV) promoter (Boshart *et al.*, *Cell*, 1985, 41:521-530) and SV40 promoter (Subramani *et al.*, *Mol. Cell. Biol.*, 1981, 1:854-864) have been found to be suitable, but others can be used as well. Optionally, the NHP-encoding nucleic acid sequences used in the subject invention include a sequence encoding a signal peptide upstream of the NHP-encoding sequence, thereby permitting secretion of the NHP from a host cell. Also, various promoters may be used to limit the expression of the peptide in specific cells or tissues, such as lung cells.

The pharmaceutical composition of the present invention can include a liposome component. According to the present invention, a liposome comprises a lipid composition that is capable of fusing with the plasma membrane of a cell, thereby allowing the liposome to deliver a nucleic acid molecule and/or a protein composition into a cell. Some preferred liposomes of the present invention include those liposomes commonly used in, for example, gene delivery methods known to those of skill in the art. Some preferred liposome delivery vehicles comprise multilamellar vesicle (MLV) lipids and extruded lipids, although the invention is not limited to such liposomes. Methods for preparation of MLV's are well known in the art. According to the present invention, "extruded lipids" are lipids which are prepared similarly to MLV lipids, but which are subsequently extruded through filters of decreasing size, as described in Templeton *et al.*, *Nature Biotech.*, 1997, 15:647-652, which is incorporated herein by refcrence in its entirety. Small unilamellar vesicle (SUV) lipids can also be used in the composition and method of the present invention. Other preferred liposome delivery vehicles comprise liposomes having a polycationic lipid composition (*i.e.*, cationic liposomes). For example, cationic liposome compositions include, but are not limited to, any cationic liposome complexed with cholesterol, and without limitation, include DOTMA and cholesterol, DOTAP and cholesterol, DOTIM and cholesterol, and DDAB and

cholesterol. Liposomes of the present invention can be any size, including from about 10 to 1000 nanometers (nm), or any size in between.

A liposome delivery vehicle of the present invention can be modified to target a particular site in a mammal, thereby targeting and making use of a nucleic acid molecule of the present invention at that site. Suitable modifications include manipulating the chemical formula of the lipid portion of the delivery vehicle. Manipulating the chemical formula of the lipid portion of the delivery vehicle can elicit the extracellular or intracellular targeting of the delivery vehicle. For example, a chemical can be added to the lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the liposome fuses with particular cells having particular charge characteristics. In one embodiment, other targeting mechanisms, such as targeting by addition of exogenous targeting molecules to a liposome (*i.e.*, antibodies) may not be a necessary component of the liposome of the present invention, since effective immune activation at immunologically active organs can already be provided by the composition when the route of delivery is intravenous or intraperitoneal, without the aid of additional targeting mechanisms. However, in some embodiments, a liposome can be directed to a particular target cell or tissue by using a targeting agent, such as an antibody, soluble receptor or ligand, incorporated with the liposome, to target a particular cell or tissue to which the targeting molecule can bind. Targeting liposomes are described, for example, in Ho *et al.*, *Biochemistry*, 1986, 25: 5500-6; Ho *et al.*, *J Biol Chem*, 1987a, 262: 13979-84; Ho *et al.*, *J Biol Chem*, 1987b, 262: 13973-8; and U.S. Patent No. 4,957,735 to Huang *et al.*, each of which is incorporated herein by reference in its entirety). In one embodiment, if avoidance of the efficient uptake of injected liposomes by reticuloendothelial system cells due to opsonization of liposomes by plasma proteins or other factors is desired, hydrophilic lipids, such as gangliosides (Allen *et al.*, *FEBS Lett*, 1987, 223: 42-6) or polyethylene glycol (PEG)-derived lipids (Klibanov *et al.*, *FEBS Lett*, 1990, 268: 235-7), can be incorporated into the bilayer of a conventional liposome to form the so-called sterically-stabilized or "stealth" liposomes (Woodle *et al.*, *Biochim Biophys Acta*, 1992, 1113: 171-99). Variations of such liposomes are described, for example, in U.S. Patent No. 5,705,187 to Unger *et al.*, U.S. Patent No. 5,820,873 to Choi *et al.*, U.S. Patent No. 5,817,856 to Tirosh *et al.*; U.S. Patent No. 5,686,101 to Tagawa *et al.*; U.S. Patent No. 5,043,164 to Huang *et al.*, and U.S. Patent No. 5,013,556 to Woodle *et al.*, all of which are incorporated herein by reference in their entireties).

generally as in PCR Protocols: A Guide To Methods And Applications, Academic Press, San Diego, Calif. (1990). *In situ* (In-cell) PCR in combination with Flow Cytometry can be used for detection of cells containing specific DNA and mRNA sequences (Testoni *et al.*, *Blood*, 1996, 87:3822.)

The term "gene therapy", as used herein, refers to the transfer of genetic material (*e.g.*, DNA or RNA) of interest into a host to treat or prevent a genetic or acquired disease or condition phenotype. The genetic material of interest encodes a product (*e.g.*, a protein, polypeptide, peptide, or functional RNA) whose production *in vivo* is desired. For example, the genetic material of interest can encode a hormone, receptor, enzyme, polypeptide or peptide of therapeutic value. For a review see, in general, the text "Gene Therapy" (Advances in Pharmacology 40, Academic Press, 1997).

Two basic approaches to gene therapy have evolved: (1) *ex vivo* and (2) *in vivo* gene therapy. In *ex vivo* gene therapy, cells are removed from a patient and, while being cultured, are treated *in vitro*. Generally, a functional replacement gene is introduced into the cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, *etc.*) and an expression system as needed and then the modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells have been shown to produce the transfected gene product *in situ*.

In *in vivo* gene therapy, target cells are not removed from the subject, rather the gene to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient. Alternatively, if the host gene is defective, the gene is repaired *in situ*. These genetically altered cells have been shown to produce the transfected gene product *in situ*.

The gene expression vector is capable of delivery/transfer of heterologous nucleic acid sequences into a host cell. The expression vector may include elements to control targeting, expression and transcription of the nucleic acid sequence in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle.

The expression vector can include a promoter for controlling transcription of the heterologous material and can be either a constitutive or inducible promoter to allow selective transcription. The expression vector can also include a selection gene.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook *et*

atmosphere of 5 % CO_2/95 % air. Cells were subcultured weekly and used between passages 9 and 22. Experiments were repeated with primary NHBE obtained from CLONETICS (Walkersville, MD) from pooled donors. These cells were cultured in BEBM medium supplied by the vendor and supplemented with 10 % fetal bovine serum and a mix of growth factors without antibiotics. Cells were grown at 37°C in 5 % CO_2/95 % and used between passages 3 and 9.

Expression plasmids and transfection. The construction of plasmid encoding NHP_{73-102} has been described previously (Kumar, M *et al.*, *J Allergy Clin Immunol.*, 2002, 110:879-82). For transfection of epithelial cells, cells at 60% confluence (log phase) were transfected 4 hr at 37°C with plasmid DNA (1 μg per 10^6 cells) complexed with lipofectamine (GIBCOBRL Life Technologies). Complete medium was then added to the cultures and the cells were incubated at 37°C for 24 to 48 h to allow expression of the natriuretic peptides.

Assay for nitric oxide. The assay for nitric oxide (NO) is based on that of Misko *et al.* (Misko, TP *et al.*, *Anal Biochem.*, 1993, 214:11-6) and measures nitrite, the stable breakdown product of NO, which is reacted with diaminonaphthalene to produce a fluorescent compound. A549 or NHBE cells were transfected with plasmid/lipofectamine complexes as described above. At specific time points, 100 μl samples of culture medium were removed and stored at -20°C. After all samples were taken, they were cleared by centrifugation and 10 μl of a freshly prepared solution of 0.02 mg/ml diaminonaphthalene was added to each tube, shaken, and allowed to react for 10 min at room temperature. The reaction was stopped by addition of 30 μl of 0.5 M NaOH and the fluorescence of the samples was read using a quartz microcuvet (3 mm path length) in a JASCO spectrofluorometer with excitation at 365 nm and emission at 409 nm. Nitrite standards were run in the same medium as the experimental samples to generate a standard curve which was used to calibrate the readings. As a positive control, one set of wells was incubated with 1 μM calcium ionophore, A23187 (SIGMA).

B. Results

NO is a bronchodilator and Ca^{++}-calmodulin binding activates the constitutive form of nitric oxide synthase (cNOS) in epithelial cells (Howarth, P.H. *et al.*, *Int Arch Allergy Immunol.*, 1995, 107:228-30). To determine whether the increased intracellular Ca^{++} seen in NHP_{73-102}-transfected cells affects nitric oxide (NO) levels, aliquots of the medium

were removed before the Ca^{++} assay and mixed with diaminonaphthalene which reacts with nitrite (from the reaction of NO and water) to produce a fluorescent compound. NO generation was significantly higher in cells expressing NHP_{73-102} (Fig. 3A and B). To verify that NO production was due to the constitutive NOS, one aliquot of cells was incubated during the expression phase with 1 mM N_{ω}-nitro-L-arginine methyl ester, an arginine analog that blocks cNOS production of NO. The enhanced NO generation was inhibited by pretreatment of the cells with N-nitro-L-arginine methyl ester, which blocks cNOS activity (Fig.3C). Airway smooth muscle hypertrophy and hyperplasia are important determinants of airway remodeling and bronchial responsiveness in asthma. NHP_{73-102} appears to act on epithelial cells to produce NO via constitutive NOS, which in turn controls bronchial hyperreactivity and proliferation of airway smooth muscle cells.

Example 5—pNHP induces anti-inflammatory response in the lung by decreasing NFkB activation of epithelial cells

A. Materials and Methods

Luciferase reporter assay for NFκB activation. A549 and NHBE cells were grown to about 60 % confluence in 12-well culture plates and transfected using LIPOFECTAMINE 2000 (INVITROGEN, Carlsbad CA). Cells were transfected with a luciferase construct under the control of an NFκB-activatable promoter (MERCURY PROFILING SYSTEM, CLONTECH, Palo Alto CA) and pLacZ as a normalization control either with or without pANP. Relative amounts of plasmid DNA and lipofectamine reagent were optimized for NHBE cells and cells were transfected for 4 h in serum-free DMEM without antibiotics at 37°C. After transfection, DMEM with 10 % FBS was added and cells were incubated for 24 to 48 h at 37°C. Cells were harvested at specific time points and lysates were assayed for luciferase activity using the DUAL LUCIFERASE Assay System (PROMEGA, Madison WI) read in an MLX microplate luminometer (DYNEX TECHNOLOGIES, Chantilly VA). Transfection efficiencies were normalized by measuring β-galactosidase activity.

Statistical analysis. Experiments were repeated a minimum of three times and data are expressed as means ± SEM. Pairs of groups were compared through the use of Student's *t* tests. Differences between groups were considered significant at $p \leq 0.05$.

B. Results

I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on May 11, 2009.

/GLENNPLADWIG/
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AMENDMENT UNDER 37 CFR §1.111
Examining Group 1633
Patent Application
Docket No. USF.183XC1
Serial No. 10/526,584

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Qian Janice Li, M.D.
Art Unit	:	1633
Applicant	:	Shyam S. Mohapatra
Serial No.	:	10/526,584
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For	:	Materials and Methods for Treatment of Allergic Diseases

MS AMENDMENT
Commissioner for Patents
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AMENDMENT UNDER 37 CFR §1.111

Applicant requests that the period for response in the above-identified patent application be extended one month through and including May 12, 2009, the fees for which have been paid at the time this Amendment was filed.

In response to the Office Action dated January 12, 2009, please amend the above-identified application as follows:

In the Specification

Please replace the paragraph found on page 7, lines 7-8 of the specification with the following paragraph:

SEQ ID NO:5 is the amino acid sequence of cloned mouse $pNHP_{73-102}$: ^{73}GSPWDPSDRSALLKSKLRALLAGPRSLRRS^{102}.

Please replace the paragraph at page 11, line 2 of the specification with the following paragraph:

SEQ ID NO:18 is the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 5:

ggc agc ccc tgg gac ccc tcc gat aga tct gcc ctc ttg aaa agc aaa ctg agg gct ctg ctc gct ggc cct cgg agc cta cga aga tcc

Please delete existing pages 1-9 of the Sequence Listing and insert the attached new pages 1-9 of the Sequence Listing.

In the Claims

1-12 (Canceled).

13 (Currently Amended). A pharmaceutical composition comprising:

a nucleic acid sequence encoding a natriuretic hormone peptide~~, or a biologically active portion of the natriuretic hormone peptide, wherein the natriuretic hormone peptide comprises~~ comprising an amino acid sequence comprising SEQ ID NO:5 or a homolog ~~thereof~~ of SEQ ID NO: 5 having at least one conservative amino acid substitution, and an operably linked promoter sequence; and a pharmaceutically acceptable carrier.

14-19 (Canceled).

20 (Currently Amended). An expression vector comprising:

a nucleic acid sequence encoding a natriuretic hormone peptide comprising ~~a biologically active portion of an amino acid sequence comprising the amino acid sequence of SEQ ID NO:5, SEQ ID NO:6, or a homolog of SEQ ID NO: 5 or SEQ ID NO: 6~~ an amino acid sequence comprising SEQ ID NO: 5 or a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution, or comprising an amino acid sequence of SEQ ID NO: 6; and an operably linked promoter sequence.

21 (Previously Presented). The expression vector of claim 20, wherein the natriuretic hormone peptide comprises an amino acid sequence comprising SEQ ID NO: 6.

22 (Canceled).

23 (Currently Amended). An isolated cell comprising a nucleic acid sequence encoding a natriuretic hormone peptide~~, the peptide comprising a biologically active portion of an amino acid sequence comprising the amino acid sequence of~~ comprising an amino acid sequence comprising SEQ ID NO:5 or a homolog of SEQ ID NO: 5 having at least one conservative amino acid

substitution, or comprising SEQ ID NO: 6~~, or a homolog of SEQ ID NO: 5 or SEQ ID NO: 6,~~; and an operably linked promoter sequence.

24 (Previously Presented). The isolated cell of claim 23, wherein the natriuretic hormone peptide comprises an amino acid sequence comprising SEQ ID NO: 6.

25 (Currently Amended). An isolated nucleic acid molecule comprising a nucleic acid sequence encoding ~~a biologically active portion of an amino acid sequence consisting essentially of: SEQ ID NO: 5, SEQ ID NO: 6, or a homolog of SEQ ID NO: 5 or SEQ ID NO: 6~~ an amino acid sequence comprising SEQ ID NO: 5 or a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution, or comprising SEQ ID NO: 6.

26 (Canceled).

27 (Previously Presented). The pharmaceutical composition of claim 13, further comprising a chitosan.

28 (Currently Amended). An expression vector comprising: a nucleic acid sequence encoding a natriuretic hormone peptide~~, or a biologically active portion of the natriuretic hormone peptide, wherein the natriuretic hormone peptide comprises~~ comprising an amino acid sequence comprising SEQ ID NO: 5 or a homolog ~~thereof~~ of SEQ ID NO: 5 having at least one conservative amino acid substitution, and an operably linked promoter sequence.

29 (Currently Amended). An isolated cell comprising a nucleic acid sequence encoding a natriuretic hormone peptide~~, or a biologically active portion of the natriuretic hormone peptide, wherein the natriuretic hormone peptide comprises~~ comprising an amino acid sequence comprising SEQ ID NO: 5 or a homolog ~~thereof~~ of SEQ ID NO: 5 having at least one conservative amino acid substitution, and an operably linked promoter sequence.

30 (Currently Amended). An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a natriuretic hormone peptide~~, or a biologically active portion of the natriuretic hormone peptide, wherein the natriuretic hormone peptide comprises~~ comprising an amino acid sequence comprising SEQ ID NO: 5 or a homolog ~~thereof~~ of SEQ ID NO:5 have at least one conservative amino acid substitution.

31-42 (Canceled).

43 (Previously Presented). The pharmaceutical composition of claim 13, further comprising a liposome.

44 (Canceled).

45 (Previously Presented). The expression vector of claim 28, wherein the expression vector is a DNA plasmid.

46-47 (Canceled).

48 (Previously Presented). The pharmaceutical composition according to claim 13, wherein the natriuretic hormone peptide consists of the amino acid sequence of SEQ ID NO: 5.

49 (Previously Presented). The expression vector according to claim 28, wherein the natriuretic hormone peptide consists of the amino acid sequence of SEQ ID NO: 5.

50 (Previously Presented). The isolated cell according to claim 29, wherein the natriuretic hormone peptide consists of the amino acid sequence of SEQ ID NO: 5.

51 (Previously Presented). The isolated nucleic acid sequence according to claim 30, wherein the natriuretic hormone peptide consists of the amino acid sequence of SEQ ID NO: 5.

52 (Currently Amended). The pharmaceutical composition according to claim 13, wherein the nucleic acid sequence comprises the nucleotide sequence of ~~SEQ ID NO: 12 or~~ SEQ ID NO: 18.

53 (Currently Amended). The expression vector according to claim 20, wherein the nucleic acid sequence comprises the nucleotide sequence of ~~SEQ ID NO: 12, SEQ ID NO: 13,~~ SEQ ID NO: 18[[,]] or SEQ ID NO: 19.

54 (Currently Amended). The isolated cell according to claim 23, wherein the nucleic acid sequence comprises the nucleotide sequence of ~~SEQ ID NO: 12, SEQ ID NO: 13,~~ SEQ ID NO: 18[[,]] or SEQ ID NO: 19.

55 (Currently Amended). The isolated nucleic acid sequence according to claim 25, wherein the nucleic acid sequence comprises the nucleotide sequence of ~~SEQ ID NO: 12, SEQ ID NO: 13,~~ SEQ ID NO: 18[[,]] or SEQ ID NO: 19.

56 (Currently Amended). The expression vector according to claim 28, wherein the nucleic acid sequence comprises the nucleotide sequence of ~~SEQ ID NO: 12 or~~ SEQ ID NO: 18.

57 (Currently Amended). The isolated cell according to claim 29, wherein the nucleic acid sequence comprises the nucleotide sequence of ~~SEQ ID NO: 12 or~~ SEQ ID NO: 18.

58 (Currently Amended). The isolated nucleic acid sequence according to claim 30, wherein the nucleic acid sequence comprises the nucleotide sequence of ~~SEQ ID NO: 12 or~~ SEQ ID NO: 18.

59 (Currently Amended). A pharmaceutical composition comprising:

a nucleic acid molecule comprising a nucleic acid sequence encoding a natriuretic hormone peptide~~, or a biologically active portion of the natriuretic hormone peptide, wherein the natriuretic hormone peptide comprises an~~ comprising an amino acid sequence comprising SEQ ID NO: 6 ~~or a~~

~~homolog thereof~~, and an operably linked promoter sequence; and a pharmaceutically acceptable carrier.

60 (Previously Presented). The pharmaceutical composition of claim 59, further comprising a liposome.

61 (Previously Presented). The pharmaceutical composition according to claim 59, wherein the natriuretic hormone peptide consists of the amino acid sequence of SEQ ID NO: 6.

62 (Currently Amended). The pharmaceutical composition according to claim 59, wherein the nucleic acid sequence comprises the nucleotide sequence of ~~SEQ ID NO: 13 or~~ SEQ ID NO: 19.

63 (New). The pharmaceutical composition according to claim 13, wherein the natriuretic hormone peptide comprises the amino acid sequence of SEQ ID NO: 5.

64 (New). The expression vector according to claim 28, wherein the natriuretic hormone peptide comprises the amino acid sequence of SEQ ID NO: 5.

65 (New). The isolated cell according to claim 29, wherein the natriuretic hormone peptide comprises the amino acid sequence of SEQ ID NO: 5.

66 (New). The isolated nucleic acid molecule according to claim 30, wherein the natriuretic hormone peptide comprises the amino acid sequence of SEQ ID NO: 5.

67 (New). The expression vector according to claim 20, wherein the natriuretic hormone peptide comprises an amino acid sequence consisting of SEQ ID NO: 6.

68 (New). The isolated cell according to claim 23, wherein the natriuretic hormone peptide comprises an amino acid sequence consisting of SEQ ID NO: 6.

69 (New). The isolated nucleic acid sequence according to claim 25, wherein the amino acid sequence consists of SEQ ID NO: 6.

Remarks

Claims 13, 20, 21, 23-25, 27-30, 43, 45 and 48-62 were pending in the subject application. By this Amendment, claims 13, 20, 23, 25, 28-30, 52-59, and 62 have been amended, and new claims 63-69 have been added. The undersigned avers that no new matter is introduced by this amendment. Support for the new claims and amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 13, 20, 21, 23-25, 27-30, 43, 45 and 48-69 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

The specification is objected to because the 29-amino acid sequence of SEQ ID NO:5 ($pNHP_{73-102}$) only accounts for residues 73-101 of $pNHP_{73-102}$, *i.e.*, the residue at the 102 position of $pNHP_{73-102}$ is missing from the end of the sequence of SEQ ID NO:5. Accordingly, by this Amendment, page 7 of the specification has been amended to add a serine (Ser) residue to the carboxy-terminal end of SEQ ID NO: 5, which is the residue at position 102 of $pNHP_{73-102}$. Likewise, page 7 of the specification has been amended to add the codon for Ser (-tcc-) to SEQ ID NO: 18, which is the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 5. Pages 1-9 of the Sequence Listing have also been replaced with a new Sequence Listing with these corrections to SEQ ID NO: 5 and SEQ ID NO: 18. Support for these amendments can be found throughout the specification including, for example, nucleotides 459-461 of SEQ ID NO: 17 at page 10 of the specification as originally filed.

Claims 52-58 and 62 are objected to because they contain subject matter drawn to non-elected inventions (SEQ ID NOs: 12, 13). By this Amendment, SEQ ID NO: 12 and SEQ ID NO: 13 have been removed from the claims, thereby obviating the objection.

Claims 13, 20, 23, 25, and 28-30 are rejected under 35 USC §102(b) as anticipated by Seidman *et al.* (1984). The Examiner asserts that the Seidman *et al.* publication teaches an expression vector comprising a nucleic acid sequence encoding a natriuretic peptide hormone, which comprises "part of the instant SEQ ID NO: 5", and host cells comprising the vector. The applicant respectfully traverses this rejection.

By this Amendment, the applicant has amended independent claims 13 and 28-30 to recite that the nucleic acid sequence encodes a natriuretic hormone peptide comprising an amino acid sequence comprising SEQ ID NO: 5, or a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution. In addition, the applicant has amended independent claims 20 and 23 to recite that the nucleic acid sequence encodes a natriuretic hormone peptide comprising an amino acid sequence comprising SEQ ID NO: 5, or a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution, or comprising an amino acid sequence of SEQ ID NO: 6. Support for these amendments can be found, for example, at page 14, lines 17-28, and Table 1 of the specification.

The Seidman *et al.* publication does not teach or suggest a natriuretic hormone peptide comprising the amino acid of SEQ ID NO: 5 or a homolog thereof having at least one conservative amino acid substitution. Thus, for at least this reason, the Seidman *et al.* reference does not teach the applicant's claimed invention. As the Examiner is aware, in order to anticipate, a single reference must disclose within the four corners of the document each and every element and limitation contained in the rejected claim. *Scripps Clinic & Research Foundation v. Genentech Inc.*, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991). The Seidman *et al.* reference fails to teach or suggest every element of the applicant's claimed invention. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(b) is respectfully requested.

Claims 13, 20, 21, 23-25, 28-30, 43, 45, 59, and 60 are also rejected under 35 USC §102(b) as anticipated by Shimkets (U.S. Patent No. 6,013,630). The Examiner asserts that the Shimkets patent teaches a pharmaceutical composition comprising "residue 3 through 29 of the instant SEQ ID NO: 5, a homolog of instant SEQ ID NO: 5." The applicant respectfully traverses this rejection.

As indicated above, the applicant has amended independent claims 13 and 28-30 to recite that the nucleic acid sequence encodes a natriuretic hormone peptide comprising an amino acid sequence comprising SEQ ID NO: 5, or a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution. Additionally, the applicant has amended independent claims 20 and 23 to recite that the nucleic acid sequence encodes a natriuretic hormone peptide comprising an amino acid sequence comprising SEQ ID NO: 5, or a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution, or comprising an amino acid sequence of SEQ ID NO: 6.

Independent claim 59 has been amended to recite that the nucleic acid sequence encodes a natriuretic hormone peptide comprising an amino acid sequence comprising SEQ ID NO: 6.

SEQ ID NO: 1 of the Shimkets patent is not a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution. As is evident from the sequences presented below, the second amino acid and the last amino acid of SEQ ID NO: 5 of the subject application are serines, which are not present in SEQ ID NO: 1 of the Shimkets patent.

SEQ ID NO:5 of the subject application:
Gly-**Ser**-Pro-Trp-Asp-Pro-Ser-Asp-Arg-Ser-Ala-Leu-Leu-Lys-Ser-Lys-Leu-Arg-Ala-Leu-Leu-Ala-Gly-Pro-Arg-Ser-Leu-Arg-Arg-**Ser**

SEQ ID NO:1 of the Shimkets patent:
Gly-Pro-Trp-Asp-Pro-Ser-Asp-Arg-Ser-Ala-Leu-Leu-Lys-Ser-Lys-Leu-Arg-Ala-Leu-Leu-Ala-Gly-Pro-Arg-Ser-Leu-Arg-Arg

The Examiner also asserts that the Shimkets patent teaches "a nucleic acid encoding a ANF comprising instant SEQ ID NO: 6 (SEQ ID NO:2 of Shimkets)." The applicant submits that SEQ ID NO: 6 of the subject application is not found within SEQ ID NO: 2 of the Shimkets patent. As is evident from the sequences presented below, residue 67 of SEQ ID NO: 2 of the Shimkets patent has an aspartic acid where SEQ ID NO: 6 of the subject application has a glutamic acid.

SEQ ID NO:6 of the subject application:
Val-Ser-Asn-Thr-Asp-Leu-Met-Asp-Phe-Lys-Asn-Leu-Leu-Asp-His-Leu-Glu-Glu-Lys-Met-Pro-Val-Glu-Asp-Glu-Val-Met-Pro-Pro-Gln-Ala-Leu-Ser-Glu-Gln-Thr-**Glu**

Amino acids 31-67 of SEQ ID NO:2 of the Shimkets patent:
Val-Ser-Asn-Thr-Asp-Leu-Met-Asp-Phe-Lys-Asn-Leu-Leu-Asp-His-Leu-Glu-Glu-Lys-Met-Pro-Val-Glu-Asp-Glu-Val-Met-Pro-Pro-Gln-Ala-Leu-Ser-Glu-Gln-Thr-**Asp**

The Shimkets patent fails to teach or suggest every element of the applicant's claimed invention. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(b) is respectfully requested.

Claims 20, 21, 23-25, and 59 are rejected under 35 USC §102(b) as anticipated by Zivin *et al.* (1984). The Examiner asserts that the Zivin *et al.* publication teaches a nucleic acid sequence encoding a natriuretic hormone peptide "comprising amino acid residues of instant SEQ ID NO: 6

(see figure 1), plasmid vectors comprising the nucleic acids (pBR322), and host cells comprising the vector." The applicant respectfully traverses this rejection.

The applicant has amended independent claims 20 and 23 to recite that the nucleic acid sequence encodes a natriuretic hormone peptide comprising an amino acid sequence comprising SEQ ID NO: 5, or a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution, or comprising an amino acid sequence of SEQ ID NO: 6. As is evident from the sequences presented below, residue 67 of Fig. 1 of the Zivin *et al.* publication has an aspartic acid where SEQ ID NO: 6 of the subject application has a glutamic acid.

SEQ ID NO:6 of the subject application:
Val-Ser-Asn-Thr-Asp-Leu-Met-Asp-Phe-Lys-Asn-Leu-Leu-Asp-His-Leu-Glu-Glu-Lys-Met-Pro-Val-Glu-Asp-Glu-Val-Met-Pro-Pro-Gln-Ala-Leu-Ser-Glu-Gln-Thr-**Glu**
Figure 1 of Ziven *et al.*:
Val-Ser-Asn-Thr-Asp-Leu-Met-Asp-Phe-Lys-Asn-Leu-Leu-Asp-His-Leu-Glu-Glu-Lys-Met-Pro-Val-Glu-Asp-Glu-Val-Met-Pro-Pro-Gln-Ala-Leu-Ser-Glu-Gln-Thr-**Asp**

For at least this reason, the Zivin *et al.* publication fails to teach or suggest every element of the applicant's claimed invention. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(b) is respectfully requested.

Claims 20, 21, 23-25, 53-55, 59, and 62 are rejected under 35 USC §102(a) as being anticipated by Collins *et al.* (2002). The Examiner asserts that the Collins *et al.* publication discloses a nucleic acid sequence comprising SEQ ID NO: 19, which encodes the amino acid sequence of SEQ ID NO: 6, as well as vectors and host cells used in cloning and sequence. The applicant respectfully traverses this rejection.

The Office Action contends that the Collins *et al.* publication teaches the aforementioned nucleic acid sequence of SEQ ID NO: 19. This contention is respectfully traversed. Initially, it is noted that the Office has not identified where in the Collins *et al.* publication an alleged anticipatory teaching is to be found. No figure or sequence within the Collins *et al.* publication is cited by the Office Action in support of the contention that the Collins *et al.* publication teaches the nucleic acid sequence of SEQ ID NO: 19. Furthermore, the applicant has carefully reviewed the Collins *et al.* publication and finds no teaching of the nucleic acid sequence of SEQ ID NO: 19, an expression vector comprising the nucleic acid sequence, an isolated cell comprising the nucleic acid sequence,

or a pharmaceutical composition comprising the nucleic acid sequence, as recited in the rejected claims. Thus, for at least this reason, the Collins *et al.* publication does not anticipate claims 20, 21, 23-25, 53-55, 59, and 62.

In the event that the Office maintains this rejection, the applicant respectfully requests that the Office, in the interest of compact prosecution, identify on the record and with specificity sufficient to support a *prima facie* case of anticipation, where in the Collins *et al.* publication the claimed subject matter is alleged to be taught. Furthermore, to the extent that the Office asserts that the claimed subject matter is inherent in the Collins *et al.* publication, if such an assertion is made, the applicant notes that the presence of inherent matter must be grounded on more than speculation, it must be a certainty. *Ethyl Molded Product Co. v. Betts Package Inc.*, 9 USPQ 2d 1001, 1032-1033 (I.D.KY 1988).

Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(a) is respectfully requested.

Claims 13 and 27 are rejected under 35 USC §103(a) as being obvious over Shimkets (U.S. Patent No. 6,013,630), in view of Nicolaas *et al.* (1996). The applicant respectfully traverses this rejection.

The Examiner asserts that the Shimkets patent teaches "a pharmaceutical composition comprising a nucleic acid sequence encoding a natriuretic hormone peptide (SEQ ID NO: 1), which is a homolog of instant SEQ ID NO: 5." As indicated above, the applicant has amended independent claim 13 to recite that the nucleic acid sequence encodes a natriuretic hormone peptide comprising an amino acid sequence comprising SEQ ID NO:5, or a homolog of SEQ ID NO:5 having at least one conservative amino acid substitution. SEQ ID NO: 1 of the Shimkets patent is not a homolog of SEQ ID NO: 5 having at least one conservative amino acid substitution. As is evident from the sequences presented below, the second amino acid and the last amino acid of SEQ ID NO: 5 of the subject application are serines, which are not present in SEQ ID NO: 1 of the Shimkets patent.

SEQ ID NO:5 of the subject application:

Gly-**Ser**-Pro-Trp-Asp-Pro-Ser-Asp-Arg-Ser-Ala-Leu-Leu-Lys-Ser-Lys-Leu-Arg-Ala-Leu-Leu-Ala-Gly-Pro-Arg-Ser-Leu-Arg-Arg-**Ser**

SEQ ID NO:1 of the Shimkets patent:

Gly-Pro-Trp-Asp-Pro-Ser-Asp-Arg-Ser-Ala-Leu-Leu-Lys-Ser-Lys-Leu-Arg-Ala-Leu-Leu-Ala-Gly-Pro-Arg-Ser-Leu-Arg-Arg

The Nicolaas *et al.* publication is cited for teaching chitosan can be used to enhance small peptide drug delivery. The Nicolaas *et al.* publication does not cure the deficiencies of the Shimkets patent.

The combination of the Shimkets patent and the Nicolaas *et al.* publication fail to teach or suggest every element of the applicant's claimed invention. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §103(a) is respectfully requested.

In view of the foregoing remarks and amendments to the claims, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

/GLENNPLADWIG/

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Gainesville, FL 32614-2950

GPL/ml/jnw

Attachments: Submission of Sequence Listing and Statement
Replacement Sequence Listing on paper (pages 1-9)

I hereby certify that this correspondence is being electronically transmitted to the United States Patent and Trademark Office on May 11, 2009.

/GLENNPLADWIG/
Glenn P. Ladwig, Patent Attorney, Reg. No. 46,853

SUBMISSION OF SEQUENCE LISTING
UNDER 37 CFR §§1.821-1.825
Patent Application
Docket No. USF.183XC1
Serial No. 10/526,584

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Qian Janice Li, M.D.
Art Unit	:	1633
Applicant	:	Shyam S. Mohapatra
Serial No.	:	10/526,584
Filed	:	October 11, 2005
Conf. No.	:	4246
For	:	Materials and Methods for Treatment of Allergic Diseases

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUBMISSION OF SEQUENCE LISTING
AND STATEMENT UNDER 37 C.F.R. §§1.821-1.825

Sir:

Transmitted herewith is a replacement Sequence Listing under 37 CFR §§1.821 through 1.825 for the above-identified patent application.

The Sequence Listing is submitted in computer readable format and on paper. I hereby certify that the paper and computer readable copies contain the same information and that no new material is added by this submission.

The Commissioner is hereby authorized to charge any fees under 37 CFR 1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Respectfully submitted,

/GLENNPLADWIG/

Glenn P. Ladwig
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Fax No.: 352-372-5800
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Gainesville, FL 32614-2950

GPL/jnw
Attachments: Sequence listing on paper and in computer readable format

SEQUENCE LISTING

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Mohapatra, Shyam

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